

**AMENDMENTS TO THE CLAIMS:**

Please add new claims 96-132 as follows:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (previously presented) A method for eliciting modification of a selected RNA target in a cell comprising:
  - (a) providing a single-stranded RNA-like polynucleotide hybridizable with said RNA target;
  - (b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and
  - (c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.
2. (original) The method of claim 1 wherein said modification of the RNA target occurs in the cell's nucleus.
3. (original) The method of claim 1 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.
4. (original) The method of claim 1 wherein the RNase III polypeptide is a human RNase III polypeptide.
5. (original) The method of claim 1 wherein modification of the selected RNA target is cleavage of the RNA target.

6. (original) The method of claim 1 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.
7. (original) The method of claim 6 wherein the polypeptide comprising an RNase III domain present in enriched amounts is overexpressed or exogenously added.
8. (original) The method of claim 1 wherein the polypeptide comprising an RNase III domain is a purified RNase III polypeptide.
9. (original) The method of claim 1 wherein the RNA-like polynucleotide has a modification at the 2' position of at least one sugar.
10. (original) The method of claim 1 wherein step (c) is performed within a cell.
11. (original) The method of claim 1 wherein step (b) is performed within a cell.
12. (original) The method of claim 1 wherein step (b) is performed outside a cell.
13. (original) The method of claim 1 wherein at least one furanosyl moiety of the RNA-like polynucleotide is a ribofuranosyl moiety.
14. (original) The method of claim 13 wherein a majority of the furanosyl moieties of the RNA-like polynucleotide are ribofuranosyl moieties.
15. (original) A method for promoting gene silencing in a cell comprising providing to the cell, in an amount effective to promote said gene silencing, a polypeptide comprising an RNase III domain.

16. (original) The method of claim 15 wherein said promotion of gene silencing occurs in the cell's nucleus.
17. (original) The method of claim 15 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.
18. (original) The method of claim 15 wherein the RNase III polypeptide is a human RNase III polypeptide.
19. (original) The method of claim 15 wherein the RNase III polypeptide is exogenously added.
20. (original) The method of claim 15 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.
21. (original) The method of claim 15 wherein said RNase III polypeptide is a purified RNase III polypeptide.
22. (original) The method of claim 15 wherein said RNase III polypeptide is expressed by an exogenously added vector encoding said RNase III polypeptide.
23. (original) The method of claim 15 wherein said cell is a mammalian cell.
24. (original) The method of claim 15 wherein said cell is a human cell.
25. (original) A method for promoting gene silencing in a cell comprising enriching the amount or activity of RNase III polypeptide in said cell to a level effective to promote said gene silencing.

26. (original) The method of claim 25 wherein said promotion of gene silencing occurs in the cell's nucleus.
27. (original) The method of claim 25 wherein said enriching is by exogenous addition of RNase III polypeptide.
28. (original) The method of claim 27 wherein said exogenously added RNase III polypeptide is a purified RNase III polypeptide.
29. (original) The method of claim 25 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.
30. (original) The method of claim 25 wherein said enriching is by addition of a vector encoding the RNase III polypeptide.
31. (original) The method of claim 25 wherein said cell is a mammalian cell.
32. (original) The method of claim 25 wherein said cell is a human cell.
33. (previously presented) A method for promoting gene silencing of a gene in a cell comprising:
- (a) providing to said cell a single-stranded polynucleotide hybridizable with a target RNA encoded by a selected gene whose expression is to be silenced;
  - (b) hybridizing said polynucleotide and said target RNA to form a polynucleotide-target duplex; and
  - (c) contacting said duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the polypeptide comprising an RNase III domain, and silencing of the gene thereby.

34. (original) The method of claim 33 wherein said promotion of gene silencing occurs in the cell's nucleus.
35. (original) The method of claim 33 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.
36. (original) The method of claim 33 wherein the RNase III polypeptide is a human RNase III polypeptide.
37. (original) The method of claim 36 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% homology to SEQ ID NO: 2.
- 38-39. (cancelled).
40. (original) The method of claim 33 wherein the polynucleotide is an antisense oligonucleotide.
41. (original) The method of claim 33 wherein the polynucleotide is an RNA-like polynucleotide.
42. (original) The method of claim 33 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.
43. (original) The method of claim 42 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.
44. (original) The method of claim 33 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.

45. (original) The method of claim 44 wherein the polynucleotide has a modification at the 2' position of at least one sugar.
46. (original) The method of claim 33 wherein the RNase III polypeptide is present in enriched amounts.
47. (original) The method of claim 46 wherein the RNase III polypeptide present in enriched amounts is overexpressed or exogenously added.
48. (original) The method of claim 46 wherein the RNase III polypeptide is a purified RNase III polypeptide.
49. (original) The method of claim 46 wherein said enriching is by addition of a vector encoding said RNase III polypeptide.
50. (original) The method of claim 46 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.
51. (original) The method of claim 33 wherein said cell is a mammalian cell.
52. (original) The method of claim 33 wherein said cell is a human cell.
53. (original) The method of claim 33 wherein said polynucleotide-target RNA duplex forms inside the cell.
54. (original) The method of claim 33 wherein said polynucleotide-target RNA duplex forms outside the cell.

55. (previously presented) A method for inhibiting the expression of a gene in a cell comprising providing to said cell an agent effective to elicit RNase III modification of double-stranded RNA in the cell, wherein the agent, when a polynucleotide, is single-stranded.

56. (original) The method of claim 55 wherein said inhibition of gene expression occurs in the cell's nucleus.

57. (original) The method of claim 55 wherein said agent is a nucleic acid which is hybridizable with an RNA encoded by the gene whose expression is to be inhibited.

58. (original) The method of claim 55 wherein said RNase III modification is RNase III cleavage.

59-60. (cancelled).

61. (previously presented) The method of claim 55 wherein the agent is an antisense oligonucleotide.

62. (previously presented) The method of claim 55 wherein the agent is an RNA-like polynucleotide.

63. (previously presented) The method of claim 55 wherein the agent is a polynucleotide and wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.

64. (original) The method of claim 63 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

65. (previously presented) The method of claim 55 wherein the agent is a polynucleotide having at least one modification of the base, sugar or internucleoside linkage.

66. (original) The method of claim 65 wherein the polynucleotide has a modification at the 2' position of at least one sugar.

67. (previously presented) A method for promoting inhibition of expression of a gene in a cell comprising:

(a) providing to said cell a single-stranded polynucleotide hybridizable with a target RNA encoded by the gene whose expression is to be inhibited;

(b) hybridizing the polynucleotide and the target RNA to form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions effective to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the RNase III polypeptide, and inhibition of expression of the gene thereby.

68. (original) The method of claim 67 wherein said promotion of inhibition of gene expression occurs in the cell's nucleus.

69. (original) The method of claim 67 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

70. (original) The method of claim 69 wherein the RNase III polypeptide is a human RNase III polypeptide.

71. (original) The method of claim 70 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 2.

72-73. (cancelled).



74. (original) The method of claim 67 wherein the polynucleotide is an antisense oligonucleotide.

75. (original) The method of claim 67 wherein the polynucleotide is an RNA-like polynucleotide.

76. (original) The method of claim 67 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.

77. (original) The method of claim 76 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

78. (original) The method of claim 67 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.

79. (original) The method of claim 78 wherein the polynucleotide has a modification at the 2' position of at least one sugar.

80. (original) The method of claim 67 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.

81. (original) The method of claim 80 wherein the polypeptide comprising an RNase III domain and present in enriched amounts is overexpressed or exogenously added.

82. (original) The method of claim 81 wherein the polypeptide comprising an RNase III domain and present in enriched amounts is a purified RNase III polypeptide.

83. (original) The method of claim 81 wherein said enriching is by addition of a vector encoding said polypeptide comprising an RNase III domain.

84. (original) The method of claim 67 wherein said cell is a human cell.
85. (original) The method of claim 67 wherein step (c) is performed within a cell.
86. (original) The method of claim 67 wherein step (b) is performed within a cell.
87. (original) The method of claim 67 wherein step (b) is performed outside a cell.
88. (original) A cell having enhanced RNase III activity over an activity exhibited by a second cell, said second cell not enriched with respect to the amount or activity of RNase III polypeptide.
89. (original) The cell of claim 88 wherein said enhanced RNase III activity is detectable in the cell's nucleus.
90. (original) The cell of claim 88 wherein said enhanced RNase III activity is due to overexpression of RNase III.
91. (original) The cell of claim 88 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the RNase III polypeptide.
92. (original) The cell of claim 88 wherein said enhanced RNase III activity is due to exogenously added RNase III.
93. (previously presented) A method for eliciting modification of an RNA target in a cell comprising:
- (a) providing a single-stranded RNA-like polynucleotide hybridizable with said RNA target;

(b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.

94. (original) A hybrid RNase III comprising at least one domain from a human RNase III and at least one domain from an RNase III of an organism other than human.

95. (original) The hybrid RNase III of claim 94 wherein the non-human RNase III domain is derived from an organism selected from the group consisting of *E. coli*, *S. pombe*, *C. elegans* and *S. cerevisiae*.

96. (new) A method of reducing expression of a target gene in a cell comprising:

a) incubating a dsRNA corresponding to part of the target gene with an effective amount of a composition comprising a polypeptide comprising an RNase III domain, under conditions to allow RNase III to cleave the dsRNA into siRNA; and

b) transfecting the siRNA into the cell.

97. (new) The method of claim 96, wherein the polypeptide is chimeric.

98. (new) The method of claim 96, further comprising isolating the siRNA molecules prior to transfection.

99. (new) The method of claim 96, wherein the dsRNA is 25 to 10,000 bases or basepairs in length.

100. (new) The method of claim 99, wherein the dsRNA is 25 to 5,000 bases or basepairs in length.

101. (new) The method of claim 100, wherein the dsRNA is 50 to 1,000 bases or basepairs in length.

102. (new) The method of claim 101, wherein the dsRNA is 100 to 200 bases or basepairs in length.

103. (new) The method of claim 96, wherein the dsRNA is obtained by transcribing each strand of the dsRNA from one or more CDNA encoding the strands in vitro; isolating the strands; and, incubating the strands under conditions that allow the strands to hybridize to their complementary strands.

104. (new) The method of claim 96, wherein dsRNA for at least a second targeted gene is included.

105. (new) A method for achieving RNA interference of a target gene in a cell using one or more siRNA molecules comprising:

- a) generating at least one double-stranded DNA template corresponding to part of the target gene, wherein the DNA template comprises an SP6, T3, or T7 promoter on at least one strand;

- b) transcribing the template, wherein either i) a single RNA strand with a complementarity region, or ii) first and second complementary RNA strands is/are created;

- c) hybridizing either the single complementary RNA strand or first and second complementary RNA strands to create a dsRNA molecule corresponding to the target gene;

- d) incubating the dsRNA molecule with a polypeptide comprising an RNase III domain, under conditions to allow cleavage of the dsRNA into at least two siRNA; and

- e) transfecting at least one siRNA into the cell.

106. (new) The method of claim 105, wherein the polypeptide is RNase III.

107. (new) The method of claim 105, wherein the polypeptide is chimeric.
108. (new) The method of claim 105, wherein multiple siRNA molecules are transfected into the cell.
109. (new) A kit for generating siRNA molecules comprising:
  - a) recombinant, prokaryotic RNase III;
  - b) RNase III buffer; and
  - c) a control nucleic acid.
110. (new) The kit of claim 109, wherein the RNase III is in an enzyme dilution buffer.
111. (new) The kit of claim 109, further comprising an SP6, T3 or T7 RNA polymerase.
112. (new) The kit of claim 111, wherein the polymerase is in an enzyme mix comprising inorganic pyrophosphatase, at least one RNase inhibitor, and about 1% CHAPS.
113. (new) The kit of claim 111, further comprising an SP6, T3, or T7 polymerase buffer.
114. (new) The kit of claim 111, further comprising ATP, CTP, GTP, and UTP.
115. (new) The kit of claim 109, wherein the RNase III buffer comprises Tris and a salt.
116. (new) The kit of claim 109, wherein the control nucleic acid is DNA and comprises an SP6, T3, or T7 promoter.
117. (new) The kit of claim 109, wherein the control nucleic acid is dsRNA.

118. (new) The kit of claim 109, wherein the control nucleic acid is a DNA template capable of being transcribed into a dsRNA.
119. (new) The kit of claim 111, further comprising RNase A.
120. (new) The kit of claim 109, further comprising a cartridge, column, or filter for isolating nucleic acids.
121. (new) The kit of claim 120, further comprising binding buffer comprising NaCl.
122. (new) The kit of claim 120, further comprising wash buffer comprising NaCl.
123. (new) The kit of claim 120, further comprising an elution solution comprising Tris and EDTA.
124. (new) A method for generating siRNA that can reduce expression of a target gene comprising incubating a dsRNA corresponding to part of the target gene with an effective amount of a composition comprising a polypeptide comprising an RNase III domain, under conditions to allow RNase III to cleave the dsRNA into siRNA.
125. (new) The method of claim 124, wherein the polypeptide is chimeric.
126. (new) The method of claim 124, further comprising isolating the siRNA molecules.
127. (new) The method of claim 124, wherein the composition further comprises an RNase III buffer comprising Tris and a salt.
128. (new) The method of claim 124, wherein the dsRNA is 25 to 10,000 bases or basepairs in length.

129. (new) The method of claim 128, wherein the dsRNA is 25 to 5,000 bases or basepairs in length.

130. (new) The method of claim 129, wherein the dsRNA is 50 to 1,000 bases or basepairs in length.

131. (new) The method of claim 130, wherein the dsRNA is 100 to 200 bases or basepairs in length.

132. (new) The method of claim 124, wherein dsRNA for at least a second targeted gene is included.